

Production of potent fully human polyclonal antibodies against Zaire Ebola virus in transchromosomal cattle

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## Abstract

Polyclonal antibodies, derived from humans or hyperimmunized animals, have been used prophylactically or therapeutically as countermeasures for a variety of infectious diseases. SAB Biotherapeutics has successfully developed a transchromosomal (Tc) bovine platform technology that can rapidly produce fully human immunoglobulins in substantial quantities against a variety of disease targets. In this study, two Tc bovines expressing high levels of human IgG were hyperimmunized with a recombinant glycoprotein (GP) vaccine consisting of the 2014 Ebola virus (EBOV)-Makona isolate. Serum collected from these hyperimmunized Tc bovines contained high titers of human IgG against EBOV GP as determined by GP specific ELISA and virus neutralization assays. Fully human polyclonal antibodies against EBOV were purified and evaluated in a mouse challenge model using mouse adapted Ebola virus (maEBOV). Intraperitoneal administration of the purified anti-EBOV IgG (100 mg/kg) to BALB/c mice one day after lethal challenge with maEBOV resulted in 90% protection; whereas, 100 % the control animals succumbed. The results show that hyperimmunization of Tc bovines with EBOV GP can elicit potent neutralizing and protective human IgG antibodies rapidly and in large quantities.

## Introduction

Ebola virus (EBOV) belongs to *Ebolavirus* genus of the family *Filoviridae* and infects both humans and non-human primates (NHP) causing severe hemorrhagic fevers with symptoms of disease including sudden onset of fever, chills, headache, and anorexia followed by sore throat, vomiting, diarrhea, hemorrhaging, and the appearance of a petechial rash<sup>1-3</sup>. Filoviruses are categorized as priority class A pathogens by the Centers for Disease Control (CDC) and the National Institutes of Health (NIH), as they present a clear biological warfare threat with mortality approaching 60-90% for certain viral subtypes<sup>4,5</sup>. The most recent outbreak of EBOV in Western Africa has clearly demonstrated that filoviruses pose a huge threat to public health worldwide. Additionally, there remains concern about the deliberate misuse of these naturally occurring or mutated pathogens, posing a very high risk to national security. Presently, there are no licensed prophylactic or therapeutic countermeasures for EBOV infections in humans. Effective countermeasures that include vaccines, antivirals, and other prophylactic and therapeutic treatments that can be rapidly produced in clinically relevant quantities are top research priorities.

In laboratory studies, treatment with multiple doses of KZ52, a human monoclonal antibody (mAb) derived from an EBOV survivor, prevented Ebola virus disease (EVD) in guinea pigs<sup>6</sup>; however, follow up studies in non-human primates (NHPs) failed to show measurable protection<sup>7</sup>. More recently, studies have demonstrated that purified macaque polyclonal IgG from convalescent monkey plasma, when given

up to 48 hours post exposure, provides complete protection of NHP against filovirus challenge<sup>8</sup>. ZMapp (a cocktail of three humanized monoclonal antibodies produced in transgenic tobacco leaves) recently demonstrated a high level of protection in primates (NHPs 3 to 5 days after lethal challenge<sup>9-11</sup>).

Convalescent plasma and ZMapp have been used in a small number of humans with EBOV, but logistical and production limitations have prevented widespread use<sup>12-14</sup>.

Current immunoglobulin products, such as human intravenous immunoglobulin (IVIG), monoclonal antibodies, and animal-derived polyclonal antibodies (pAbs), have known limitations. For example, human pAb products require large volumes of plasma from many convalescent human donors with confirmed high titers to make a commercial product<sup>15, 16</sup>. Although animal-derived pAbs could be an alternative, they typically have very high reactogenicity since animal-derived antibody products are foreign proteins in humans. This can cause a variety of adverse effects such as severe allergic reactions (anaphylaxis)<sup>17, 18</sup>. To avoid serious side effects, animal antibodies are usually processed into smaller F(ab) or F(ab')<sub>2</sub> fragments, but this often reduces their half-life and potency. Monoclonal antibodies can be humanized or chimerized to human Fc fragments to avoid this, however, they are directed against a single epitope that may be subject to rapid mutational escape. This has led to the development of oligoclonal cocktails, but like monoclonal products, there are difficulties developing and producing enough of the product in a timely manner to assist in an outbreak scenario. It is clear that an innovative and rapid approach that combines the good safety profile of human polyclonal antibody products with the high neutralizing antibody activity derived from hyperimmune animals is needed.

To address these limitations, SAB Biotherapeutics (SAB) has developed a Transchromosomal (Tc) bovine in which the bovine immunoglobulin genes have been knocked out and a human artificial chromosome (HAC) containing the full germ line sequence of human immunoglobulin has been inserted allowing the Tc bovines to produce fully human antibodies<sup>19-22</sup>. Like traditional animal systems used to produce polyclonal antibodies, Tc bovines can be hyperimmunized over a long period of time with vaccines containing strong adjuvants and/or immune stimulators.

This Tc bovine system was recently used to produce high neutralization titer anti-hantavirus polyclonal human IgG. This product was protective in two animal models of lethal hantavirus disease<sup>30</sup>. The therapeutic efficacy of anti-hantavirus human polyclonal antibody clearly demonstrated proof-of-concept that it was possible to produce a candidate anti-viral biologic rapidly, and at high and scalable levels.

In this study, two Tc bovines were hyperimmunized with a vaccine containing nanoparticles comprised of recombinant full length EBOV/Makona GP. Fully human pAbs against EBOV with high titers in both GP-specific ELISA and virus neutralization assays were generated, purified, and evaluated *in vitro* with virus neutralization tests and *in vivo* using a mouse lethal model challenged with maEBOV.

It was shown that intraperitoneal (i. p.) administration of the purified fully human IgG (100 mg/kg) from Tc bovine plasma collected post 2<sup>nd</sup> vaccination was sufficient to protect BALB/c mice from lethal challenge with maEBOV). These data demonstrate that the Tc bovine system can be used as a countermeasure to rapidly, and in large quantities, produce fully human antibodies to treat against Ebola virus, and this type of antibody product can be further evaluated in a well-established non-human primate lethal challenge model for its therapeutic efficacy.

## **Results**

### **Production of human antibodies in Tc bovine vaccinated with a vaccine containing recombinant EBOV GP**

#### **Immunization schedule and sample collection**

Two Tc bovines (ID #2134 and #2136) were subcutaneously immunized with EBOV/Makona recombinant GP formulated with SAB's proprietary adjuvant (SAB-adj-1) that has been demonstrated to produce high antibody titers in cattle (21). A total of 8 boosts were conducted over a 4-week interval. For the first 4 boosts, a dose of 2 mg EBOV-GP administered (IM) was used, subsequently a higher dose of 5 mg of EBOV-GP (administered SQIM) was used for the fifth through eighth boosting. Serum was collected from each Tc bovine prior to vaccination, and 8-10 days post each boost and was evaluated using ELISA titer assay and virus neutralization tests. Large volumes of plasma were collected from each Tc bovine prior to immunization and on days 8-14 post each vaccination (V) between V2 and V8.

#### **Serum antibody titer as determined by ELISA**

To evaluate the antigen-specific IgG response in Tc bovines following the immunization with EBOV/Makona recombinant GP (rGP) vaccine, EBOV/Makona rGP specific ELISAs were performed with the serum samples (Figure 1A). rGP-specific ELISA titers increased considerably after the second boost in both Tc bovines. Interestingly, the antibody titer was slightly increased in Tc bovine #2314 but decreased in Tc bovine #2326 following the third boost. Antibody titers continued to moderately decline in both vaccinated Tc bovines at the fourth boost. Given this phenomena, and to determine if rGP-specific antibody titers could be boosted, rGP vaccine containing 5 mg rGP was administered from the fifth to eighth vaccinations. Following these later 5 mg immunizations, it was observed that there was a trend towards increased ELISA titers that were maintained high levels from V5 to V8. Fully human antibody against EBOV was purified as described in the experimental section from plasma collected from V2, V3, V3-V4 pooled plasma, and V6-V8 pooled plasma. The purified fully human antibody against EBOV was designated as SAB-139. Negative control antibody was purified from pre-vaccination Tc bovine plasma. These representative purified samples were also evaluated for ELISA titers (Figure 1B). Titers were expressed as units/mL for serum samples (Figure 1A) and units/mg of human IgG for purified

samples (Figure 1B). These results clearly show that the human antibody responses elicited in both Tc bovines specific to EBOV rGP are robust. Taken together, these data indicate that high titer human antibodies targeting EBOV GP can be generated by the hyperimmunized Tc bovines and that purified human IgG retained high titer binding activity against EBOV rGP.

### **Virus neutralizing antibody activity in serum samples**

To evaluate the neutralizing antibody responses generated in the hyperimmunized Tc bovines, both plaque reduction neutralization test (PRNT) against wild type EBOV and pseudovirion neutralization assay (PsVNA) using vesicular stomatitis virus (VSV) pseudotyped with the GP protein of EBOV<sup>17, 18</sup> were performed using serum samples collected after immunizations from each Tc bovine. Figure 2 shows PRNT<sub>80</sub> and PsVNA<sub>80</sub> titers for serum samples collected from V2 to V8. PRNT<sub>80</sub> and PsVNA<sub>80</sub> refer to the lowest IgG concentrations required to achieve 80% viral inhibition. Virus neutralization activity increased dramatically post V2 and continued to increase up to V8 for both Tc bovines except a slight decrease in V4 samples. Representative purified fully human IgG samples (see above) were also determined for virus neutralization activities by PRNT and PsVNA (Figure 2B). Data indicated that purified fully human IgG samples from different time points or pooled plasma retain potent neutralization activities against EBOV.

### **Hyperimmunization of Tc bovines with rGP vaccine promotes better antibody affinity maturation to native conformational rGP compared to denatured rGP**

In the current study, rGP proteins were used in surface plasmon resonance (SPR) to capture the real-time kinetics of antibody association and dissociation rates, which reflects the overall antibody binding avidity. In other studies, we observed that the conformation-dependent GP-specific neutralizing monoclonal antibodies bind to the rGP proteins from EBOV and Sudan virus (SUDV) in the His<sub>6</sub>-tag capture based SPR in HTG chips confirming conservation of native GP structure but these Mabs lost the binding with the amine coupled directly coated GLC chips suggesting partial denaturation of the GP protein and loss of conformational epitopes (data not shown).

The negative control Ig serum sample (prior to immunization sample, V1D0) did not show any reactivity to rGP proteins in SPR. Binding of 1 mg/mL Ig post second vaccination (V2) to native homologous EBOV rGP was low (183 RU), but increased significantly following a 3<sup>rd</sup> vaccination (V3) (Fig. 3A). These SPR data differ from the ELISA results (Fig. 1A) and may reflect binding to more non-conformational linear epitopes in the ELISA. Importantly, the SPR results are in agreement with the neutralization assays. We also explored binding to the receptor binding domain (EBOV rGP-RBD) (Fig.

3B). The pattern of binding was identical to that of the binding to EBOV-GP suggesting that significant fraction of the vaccination induced antibodies (V2-V5) were targeting epitopes mapping to the RBD, containing key protective targets. The EBOV rGP post-vaccination antibodies also showed cross-reactivity (although at lower Max RU) to the SUDV rGP in the native SPR assay at 1mg/ml Ig purified from the V2-V5 immune sera. (Figure 3C). To further investigate if repeated vaccinations with rGP promote antibody affinity maturation and lead to an increase in antibody avidity, we determined the off-rate constants of V2 and V3 Tc bovine Ig samples following binding to the native EBOV rGP and GP-RBD domains. We compared antibody binding off-rates to native rGP with partially denatured rGP to distinguish binding to conformational vs linear/denatured epitope specific antibodies as previously described<sup>23, 24</sup>. Immune Ig obtained from Tc bovines demonstrated ~5-fold increase in binding affinity (Figure 3D) with slower dissociation rate constants ( $> 10^{-3}$ /sec) following V3 compared to V2 against the native EBOV rGP. That difference in affinity was significantly diminished when partially denatured rGP was used, confirming that the high affinity binding after V3 was primarily due to conformational specific binding antibodies.

#### **Protective efficacy of purified fully human antibody against EBOV in mice**

To evaluate the protective efficacy of purified fully human antibody against EBOV, IgG was purified from plasma pools of two Tc bovines post 2<sup>nd</sup> vaccination (designated as SAB-139-V2) and administrated to BALB/c mice that were challenged with a lethal dose of ma-EBOV. In this experiment, BALB/c mice (n=10/group) received a single intraperitoneal (IP) injection of 100mg/kg of purified negative control pAbs or 100mg/kg of SAB-139-V2, 24hr or 48hr after challenge with 100 PFU ma-EBOV by the IP route. Kaplan-Meier survival analysis is shown indicating the percentage of surviving mice for 28 days post challenge (Figure 4). Nine of 10 mice receiving negative control antibody, and 10 of 10 mice receiving SAB-139-V2 48 hour post challenge died. However, nine of the 10 mice treated with SAB-139-V2 24 hours post challenge survived the lethal EBOV infection.

#### **Discussion**

Previous studies using a codon-optimized DNA vaccine expressing the EBOV GP gene showed that protective antibodies were generated and complete protection was observed in mice and monkeys challenged by EBOV<sup>28</sup>. Very recently, a vaccine containing recombinant EBOV/Makona GP nanoparticles was shown to be 100% protective in *Cynomolgus macaque* monkeys, and to generate sustained IgG anti-GP responses in baboons (Novavax, unpublished data). Here, we hyperimmunized two Tc bovines that expressed high levels of fully human IgG<sup>22</sup> with a vaccine comprised of EBOV/Makona rGP nanoparticles and SAB's proprietary adjuvant. Our goal was to validate the robust immune responses of EBOV/Makona rGP nanoparticle as observed in monkeys, and more importantly to produce large

quantities of human pAbs that would be suitable for passive immune therapy in filovirus-infected humans. Both Tc bovines in this study developed high neutralization antibody responses as measured by PRNT and PsVNA after 3<sup>rd</sup> vaccination. It is notable that higher dose of recombinant EBOV/Makona GP nanoparticles administered at the 5<sup>th</sup> vaccination further increased the level of neutralization activity and these increased neutralization antibody responses was maintained through the 8<sup>th</sup> vaccination.

Currently, a combination of VSV-PsVNA and PRNT are most commonly used assays to determine the neutralizing antibody responses to EBOV antigen/vaccine<sup>25</sup>. The results from both assays show a similar trend with virus neutralization titers increasing after each vaccination post V2, although the antibody activity measured by PRNT<sub>80</sub> is lower than that measured by PsVNA<sub>80</sub>. One possible reason for this difference is that the PsVNA measures GP-mediated particle entry, and not subsequent steps required for plaque formation (e.g., virion egress). Another possibility is that less GP-specific antibody is required to neutralize the VSV pseudovirion than EBOV virions due to virion size differences. The EBOV particles (~80 x 800 nm) are larger than VSV particles (~ 70 x 200 nm), and thus could potentially contain more GP trimers on their surface and would require a larger quantity of antibody to achieve neutralization. Similarly, the GP expression could be higher per surface unit area on EBOV than the pseudovirion, thus requiring more anti-GP antibody to achieve neutralization.

To evaluate the quality of antibody binding to native rGP and SPR based real time kinetics was performed. This approach also allowed measurements of polyclonal serum antibody dissociation rates following each vaccination, which can be used a surrogate of antibody affinity. Importantly the SPR allowed comparison of binding to conformationally intact native vs. partially denatured rGP that showed that 3<sup>rd</sup> vaccination, about 5-fold increase in antibody affinity was observed specifically to native conformation epitopes and not against linear/denatured epitopes within Ebola-GP. The SPR data was in very good agreement with the PRNT assay, demonstrating significant increase in total binding and higher affinity towards the native rGP after the 3<sup>rd</sup> -5<sup>th</sup> vaccinations. Binding to the RBD suggested targeting of key protective epitopes on the virus. The observed cross-reactivity against Sudan-GP and increase in antibody affinity following 3<sup>rd</sup> vaccination in SPR suggest that this Ig may have therapeutic potential against more than one Ebola type and could be further evaluated in animal studies.

Rodent experimentation to determine clinical efficacy is a first step in the proof of concept to identify attractive candidates to move forward into NHP experiments that more closely model EVD in humans. Although Tc bovine-derived anti-EBOV antibody provided 90% protection when administered one day post lethal challenge; no protection was afforded when the antibody was given 2 days post challenge. The reason for no protection 2 days post challenge is perplexing; however, similar effects on the timing of treatment on the capacity of anti-EBOV monoclonal antibodies have been reported. For

example, studies by others also have shown increased survival of rodents challenged with maEBOV when treatment with mouse Mabs was initiated at 1 or 2 days post infection<sup>27</sup>. Taken together, these data indicate that in mice there is only a very limited treatment window available for initiation of therapy after filovirus infection.

In summary, the data provide a proof of concept that filovirus rGP vaccine can be used in a Tc bovine production system to produce a fully human polyclonal IgG product that offers post-exposure protection against filovirus infection. Others have previously demonstrated that individual Mabs and Mab cocktails can protect mice and guinea pigs from lethal challenge with filoviruses (6, 28); however, neither of these animal models is necessarily predictive of protection in the gold standard NHP model of EBOV disease<sup>7, 8, 11</sup>. Future preclinical studies, including NHP studies, will provide additional insight into the further development of this fully human anti-EBOV antibody product produced using Tc bovine platform technology.

## **Materials and Methods**

### **Antigen preparation**

The antigen in this study was a recombinant glycoprotein (rGP) nanoparticle to the 2014 EBOV/Makona produced in insect cell lines and purified by a previously described method<sup>29</sup>.

### **Transchromosomal bovines**

Tc bovines were produced as previously described (<sup>19-22</sup>). Briefly, the Tc bovines used in this study are homozygous for triple knock-outs in the endogenous bovine immunoglobulin genes (*IGHM*<sup>-/-</sup> *IGHML1*<sup>-/-</sup> *IGL*<sup>-/-</sup>) and carry a human artificial chromosome (HAC) vector labeled as isKcHACD<sup>22</sup>. This HAC vector consists of human chromosome 14 fragment, which contains the entire human immunoglobulin heavy chain locus except that the IGHM constant region remains bovine and the key regulatory sequences were bovinized; and human chromosome 2 fragment, which contains the entire human immunoglobulin k light chain locus<sup>19-22</sup>. Tc bovines were produced by using genetically engineered cryo banked fibroblast cells as chromatin donors via a proprietary chromatin transfer (CT) procedure.

### **Tc bovine hyperimmunization and sample collection**

Two Tc bovines were immunized with EBOV/Makona recombinant GP (2014 Zaire strain, from Novavax Inc.) formulated with SAB's proprietary adjuvant SAB-adj-1. The Tc bovines were vaccinated 8



times (V1 – V8) at three- to four-week intervals. The antigen dose was 2 mg per animal for V1 to V4 and 5mg per animal for V5 to V8. Plasma and serum samples were taken at various time points before and/or after each vaccination. All protocols were approved by the SAB Biotherapeutics IACUC.

Prior to the first vaccination (V1), a volume of pre-vaccination plasma was collected from each study Tc bovine as the negative control. Up to 2.1% of body weight of hyperimmune plasma per animal per time point was collected from immunized Tc bovines on days 8, 11 and 14 post each vaccination starting from the second vaccination (V2) to the eighth vaccination (V8) for anti- EBOV fully human polyclonal antibody production. Plasma was collected using an automated plasmapheresis system (Baxter Healthcare, Autopheresis C Model 200). Plasmas were stored frozen at -20°C until purifications were performed.

### **Purification of fully human IgG**

Fully human IgG negative control was purified from plasma collected from Tc bovines prior to the first vaccination. Plasma collected from each vaccinated Tc bovine on day 8-14 following the second vaccination was the source material for purification of fully human anti-EBOV pAbs. Fully human IgG purification was performed as previously described<sup>22, 30</sup>. Briefly, frozen plasma is thawed overnight at 25°C, pooled, and pH adjusted to 4.8 with 20% acetic acid. Then the pH adjusted plasma was fractionated with caprylic acid in combination with a filtration step using a depth filter to remove non-IgG proteins. The filtrate was then adjusted to a pH of 7.5 with 1M Tris, further purified by using human IgG light chain kappa specific affinity chromatography followed by a second purification using bovine IgG heavy chain specific affinity chromatography. The purified fully human anti-EBOV IgG is in a sterile liquid containing 10 mM glutamic acid monosodium salt, 262 mM D-sorbitol, 0.05 mg/mL Tween80, pH 5.5.

### **ELISA titer**

Determination of EBOV rGP-specific human IgG antibody titers were performed in 96-well HB ELISA plates coated overnight at 4°C with 100 µL/well of 2 µg/mL recombinant EBOV/Makona GP (Novavax) in PBS. Plates were washed with PBST (PBS with 0.05% Tween 20) and blocked at room temperature (RT) for 1 hour with 1% BSA in PBS. After washing with PBST, serum samples or purified fully hIgG SAB-139 were added to the plates with serial dilution in PBST and incubated for 1 hr at RT. Following washing with PBST, plates were then added with diluted goat anti-human IgG-Fc conjugated with horseradish peroxidase (HRP) (Bethyl) and incubated for 1 hr at RT. After final washing with PBST, the bound anti- EBOV rGP antibodies were detected colorimetrically by using the TMB substrate kit. The absorbance was read in a microplate reader at 450nm. The standardized serum is assigned an endpoint

antibody titer value equivalent to the reciprocal dilution that produces a positive optical density of three times greater than the blank optical density. Antibody titers are reported in units/mL for serum samples, and in units/mg for purified IgG samples.

#### **Plaque Reduction Neutralization Test (PRNT)**

Serum samples collected from each vaccinated Tc bovine were heat inactivated at 56°C for 30 min and then an initial 1:5 dilution of the heat inactivated sera was made followed by two-fold serial dilutions. Samples were diluted in complete Eagle's minimum essential medium with Earle's salts containing 2% heat inactivated FBS and 0.05% Gentamicin and analyzed in duplicate. An equal volume of cEMEM supplemented with 10% guinea pig complement (Cedarlane) containing 100 pfu of Zaire virus was added to the sera dilutions and incubated at 37°C for 1 hour. Following incubation, Vero cell monolayers were inoculated, overlaid with agarose and incubated at 37°C. A second agarose overlay containing 5% neutral red was added 7 days (Zaire) later and plaques were counted the next day. Neutralization activity was determined to be the IgG concentration in serum or purified samples that reduced the number of plaques by 80% compared with the control wells. Purified IgG samples were measured using the same methods as for serum samples without heat treatment.

#### **Pseudovirion Neutralization Assay (PsVNA)**

Pseudovirions (PsV) that express luciferase were prepared in HEK 293T cells as previously described by Kwilas et al<sup>25</sup> using the EBOV-GP<sub>co</sub> plasmid to express the EBOV glycoprotein. Sera samples collected from the vaccinated Tc bovines were heat inactivated at 56°C for 30 min and then initial 1:10 dilutions of sera (in triplicate) were made followed by five-fold serial dilutions in complete Eagle's minimum essential medium with Earle's salts (cEMEM) containing 10% heat inactivated FBS, 10mM HEPES (pH 7.4) and 100 IU/ml penicillin, and 100 µg/ml streptomycin. An equal volume of cEMEM supplemented with 10% human complement (Sigma) containing 10<sup>5</sup> focus-forming units/mL of PsV was added to the serum dilutions and incubated overnight at 4°C. Following incubation, Vero cell monolayers seeded in flat, clear bottom black-walled 96-well plates (Corning) were inoculated with 50µl of the PsV:Tc bovine serum mixture and incubated at 37 °C for 18-24 hours. The medium was discarded, the cells were lysed, and luciferase substrate was added according to the *Renilla* Luciferase Assay System protocol (Promega #E2820). The luciferase data were acquired using a Tecan M200 Pro microplate reader. The raw data were graphed using GraphPad Prism to calculate percent neutralization. The data were fit to a four-parameter logistic curve and the PsVNA<sub>80</sub> neutralization activity was interpolated as the IgG concentration in serum or purified samples that inhibited 80% of luciferase activity. Purified IgG samples were not heat-inactivated before evaluation in the assay.

### **Binding of Tc bovine Ig to recombinant GP proteins and off-rate measurements by surface plasmon resonance (SPR)**

Steady-state equilibrium binding of pre- and post-GP vaccinated Tc bovine Ig was monitored at 25°C using a ProteOn surface plasmon resonance (BioRad). Recombinant GP (1 - 650 residues) and rGP-RBD (1-308 residues) were expressed with a polyhistidine tag at the C-terminus, and purified using Ni-NTA chromatography. The Ebola-GP proteins were coupled to a GLC sensor chip with amine coupling at pH 4.5 (partial denaturing condition) with 100 resonance units (RU) or captured on an HTG surface (native condition) at 100 RU in the test flow cells. Samples of 200 µL freshly prepared Ig preparations at 1 mg/mL and 0.1 mg/mL were injected at a flow rate of 50 µL/min (240-sec contact time) for association, and dissociation was performed over a 1200 second interval (at a flow rate of 50 µL/min). Responses from the protein surface were corrected for the response from a mock surface and for responses from a separate, buffer only injection. Monoclonal antibody 2D7 (anti-CCR5) was used as a negative control in these experiments. Binding kinetics for the Ig preparations and data analyses were calculated with BioRad ProteOn manager software.

Antibody off-rate constants, which describe the stability of the complex, i.e. the fraction of complexes that decays per second, were determined directly from the Ig preparation sample interaction with rGP protein using SPR (as described above) and calculated using the BioRad ProteOn manager software for the heterogeneous sample model. For all polyclonal Ig, it was important to demonstrate that the dissociation rates were independent of total GP-binding antibody titers. To that end, parallel dissociation curves for 1 mg/ml and 0.1 mg/mL for each post-vaccination Ig sample were established as previously described<sup>24</sup>. To improve the measurements, the off-rate constants were determined from two independent SPR runs.

### **Protective antibody activity in a virus challenge mouse model**

Female BALB/c mice aged 6 to 8 weeks were used in all challenge experiments with mouse-adapted EBOV (maEBOV) as described by Bray et al<sup>31</sup>. Briefly, mice were injected with 100 pfu of maEBOV diluted in PBS. Prior to use of maEBOV at 100pfu a dosing experiment was completed and lethality was observed to be higher at 100pfu than at the traditional 1000pfu (95% and 85% respectively). Therefore all of our challenges in BALB/c mice are completed with the more lethal 100pfu infection dose. All challenge studies involving the use of maEBOV were performed at USAMRIID in animal Biosafety level 4 laboratories. Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the

Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

Negative control pAbs and anti-EBOV pAbs were diluted in sterile PBS to the specified dosing in a volume of 0.5 mL and delivered via IP injection. Groups of 10 mice (~20 g) were administered via IP injection a single dose of negative control pAbs or anti-EBOV pAbs at a dose of 100 mg/kg. Control mice received negative control pAbs one day prior to challenge while experimental mice received anti-EBOV pAbs 24 hours or 48 hours post challenge with 1000 pfu via IP injection. Mice were observed daily for 28 days from initial challenge for signs of disease. Mouse studies were performed in the BSL-4 laboratory of USAMRIID.

#### Author Contributions

Conceived of the study: ES, GS, TL. Designed the experiments: JJ, JD, HW, JH, SK, ES, TL, GS, CY, RC, RT. Performed the experiments: SK, AK, EC, RO, SF, AH, HG, RB, JB, SK, CY. Analyzed the data: JD, HW, JH, SK, ES, TL, GS, CY, RC, RT, JJ. Contributed reagents/materials/analysis tools: JD, HW, JH, SK, ES, GS, RC, RT, JJ. Wrote the paper: JJ, HW, JD, JH, SK, GS.

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**Competing interests:** Authors from SAB Biotherapeutics, Inc. and Novavax, Inc were employees of these for-profit organizations at the time of the study and authors from these organizations have financial interests in their respective companies. The authors also have submitted patent applications related to the work which are pending. The authors have developed a lead candidate therapeutic antibody (SAB-139) from this work which would be considered a product of the company intended for further development as a commercial venture. This does not alter the authors' adherence to policies on sharing data and materials. There are no further competing interests to declare. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

### Figure legend

Figure 1. Anti- EBOV/Makona rGP titers by ELISA. (A) Sera collected from hyperimmunized two Tc bovines (#2314 and #2316) were measured for endpoint binding titers (units/mL) by rGP specific ELISA. Sera were collected 8 days post each vaccination from the second vaccination (V2) to eighth vaccination (V8). V1D0 represents the pre-immunized titers. (B) ELISA endpoint binding titers (units/mg) of purified fully human polyclonal antibody samples.

Figure 2. Virus neutralization titers or activities of anti-Ebola antibody in Tc bovine serum samples and purified fully human pAbs. (A) PRNT<sub>80</sub> and PsVNA<sub>80</sub> titers of V2-V8 serum samples. Neutralization titer, expressed as PRNT<sub>80</sub> or PsVNA<sub>80</sub>, refer to the highest serum dilution required to reduce the number of plaques by 80% or inhibit luciferase activity by 80% compared with control wells. (B) PRNT<sub>80</sub> and PsVNA<sub>80</sub> data of purified fully human pAbs. Neutralization activity, expressed as PRNT<sub>80</sub> or PsVNA<sub>80</sub>, refer to the lowest concentration of IgG required to reduce the number of plaques by 80% or inhibit luciferase activity by 80% compared with control wells.

Figure 3. Affinity of anti-EBOV antibody for rGP proteins measured by SPR. Steady-state equilibrium analysis of the binding of Tc bovine Ig preparations to rGP proteins was measured under native (rGP proteins immobilized on a HTG sensor chip) or partially denatured (rGP proteins immobilized on a GLC sensor chip) conditions. (A-C) Total binding of antibodies in Tc bovine sera to rGP proteins (EBOV-GP, SUDV-GP, and EBOV receptor binding domain) under native condition. (D) Increase in antibody affinity from V2 to V3 as measured by SPR. Antibody affinity (off-rate constants, kd) to EBOV-GP were determined. V3/V2 affinity (kd) ratios under native or partially denaturing conditions are shown.

Figure 4. ma-EBOV mouse challenge study with purified fully human polyclonal antibody against Ebola (SAB-139-V2). BALB/c mice (n=10/group) received a single intraperitoneal injection of 100 mg/kg of purified negative control pAbs or 100mg/kg of SAB-139-V2 24hr or 48hr after lethal challenge with 100 PFU ma-EBOV by intraperitoneal route. Kaplan-Meier survival analysis is shown indicating the percentage of surviving mice for 21 days post challenge.

Fig. 1

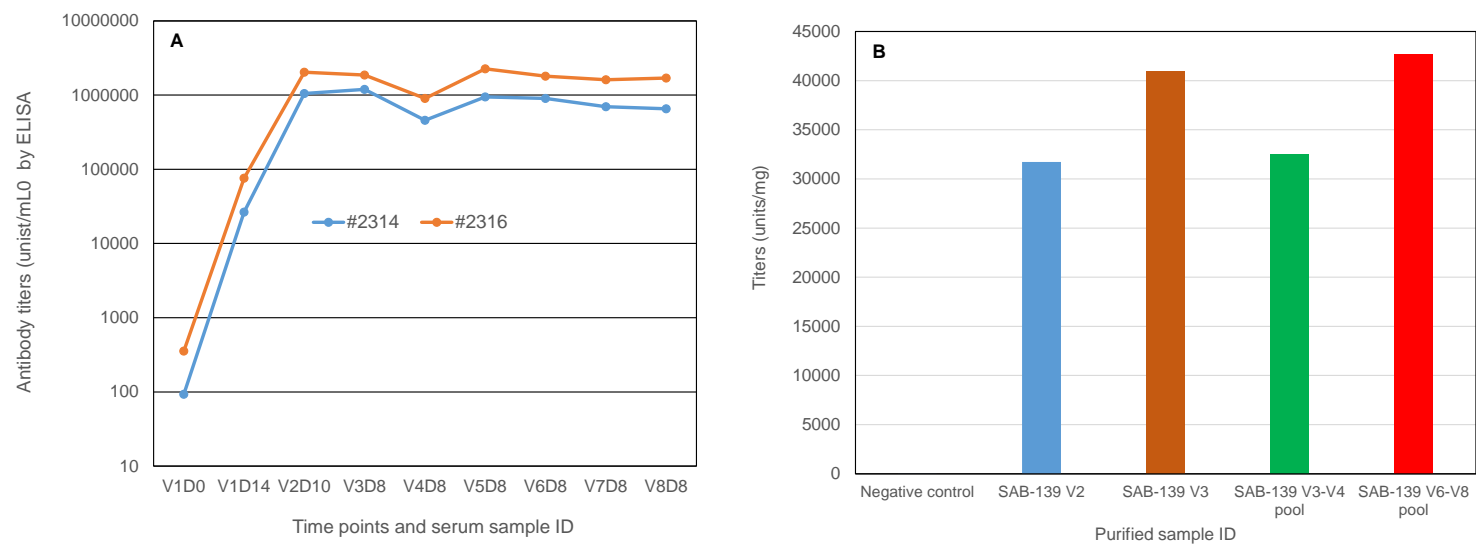


Fig. 2

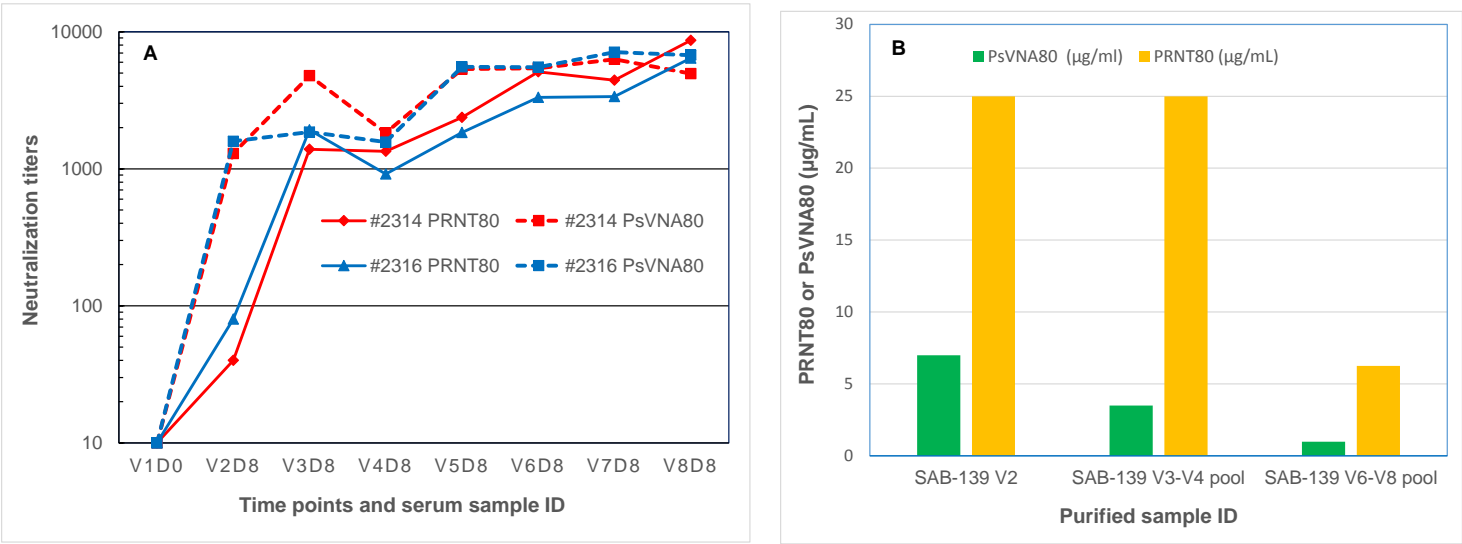


Fig. 3

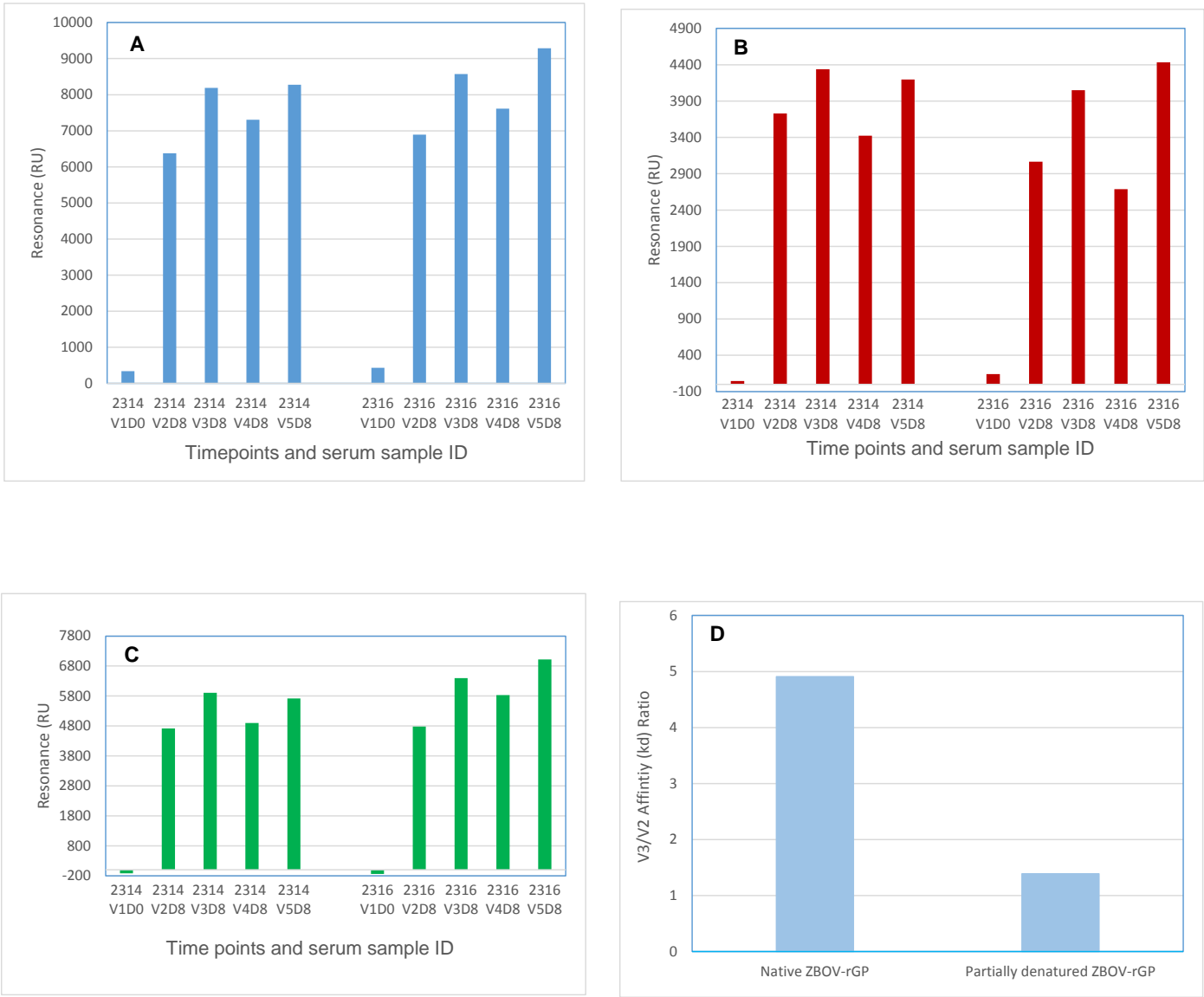
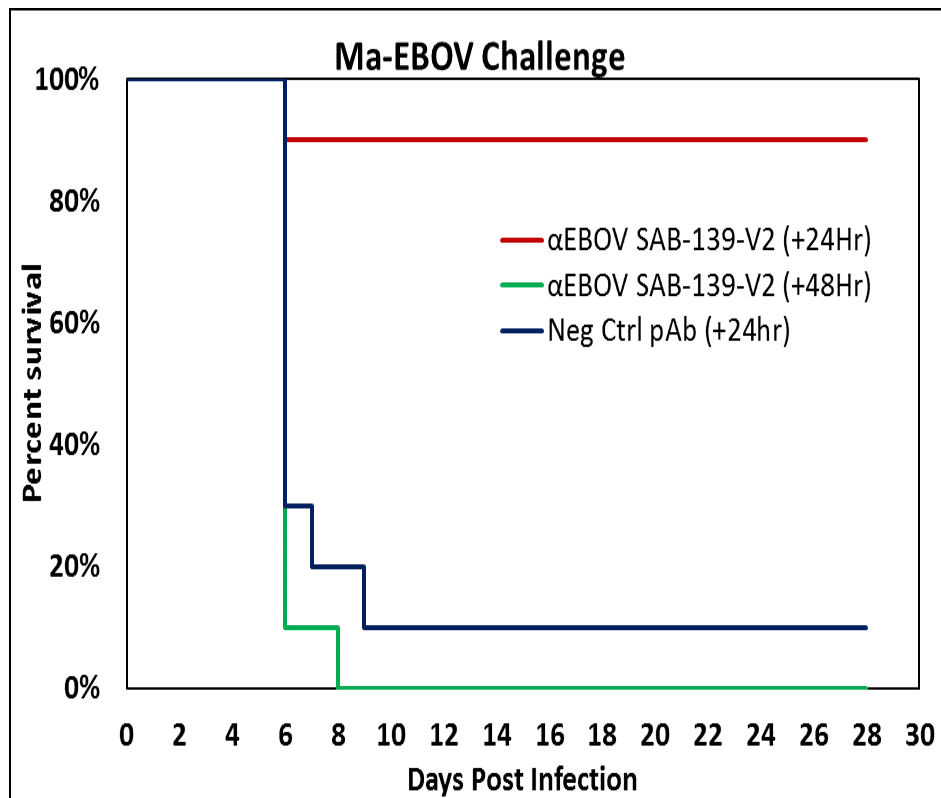




Fig. 4



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